REMARKS

Claims 50-89 currently appear in this application. The Office Action of August 5, 2003, has been carefully studied. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicants respectfully request favorable reconsideration, entry of the present amendment, and formal allowance of the claims.

Rejections under 35 U.S.C. 112

Claims 8-10, 20-30, 47 and 49 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner alleges that it is unclear what is meant by "phospholipids are derived from soy oil," the Examiner's position being that soy oil is a triglyceride of fatty acids. The Examiner suggests reciting the chemical names for E-100, S-20 and others.

The recitation in claim 20 of "adding the solute obtained in step (a)"is said to be unclear. Claim 49 is said not to be complete in itself.

This rejection is respectfully traversed. Claims 1-49 have now been rewritten as new claims 50-89. The term "soy-derived products" is well understood and commonly known and used by those versed in the art. For example, lecithin is

well recognized as crude phosphatidylcholine (PC), as opposed to purified PC. Likewise, it should be clear to one skilled in the art that the description in the instant application refers to unrefined soy oil (in the same manner that it refers to unrefined egg-yolk, etc.). This is evident from Table 1 in the specification, which shows that the source of the phospholipids may be soy oil or tomato or egg yolk with different percentages of the phospholipids in the composition of matter derived from each source.

The Examiner has requested that the claims include the chemical names of the abbreviations E-199, S-20, etc. The compositions of these substance is defined in Table 1. Since these substances refer to mixtures of substances derived from a natural product, they cannot be defined by a simple chemical name. Yet, the percentage of the phospholipids in each substance is defined. As indicated in the specification, E-100 refers to 100% PC originating from egg (E), S-020 refers to a composition of matter comprising 10% PC originating from soy oil, etc. In addition, the materials referred to in Table 1 are all commercially available, for example, from Lipoid GmbH.

It is believed that all of the newly submitted claims conform to the requirements of 35 U.S.C. 112.

Art Rejections

Claims 1-5, 7-15, 17-24, 26, 28-33, 46-47 and 49 are rejected under 35 U.S.C. 102(b) as anticipated by Meybeck.

This rejection is respectfully traversed. Claims 1-49 have been replaced by new claims 50-89. The newly submitted claims recite the following:

a. a method for preparing liposomes loaded with a water-immiscible carotenoid;

b. a formulation containing liposomes loaded with a water-immiscible carotenoid, the formulation prepared by the claimed method;

c. a pharmaceutical composition comprising the formulation prepared by the method of the present invention;

d. a method of treatment using the pharmaceutical composition of the present invention;

e. a dried composition; and

f. a kit.

Thus, the newly submitted claims are all limited by the preparative method steps recited, namely that a powder of liposome-forming lipids be dissolved in an organic solvent to a level close to saturation, **after which** at least one dry, water-immiscible carotenoid is added to obtain a suspension, which is dried to form a second dry powder. This second dry

powder is then rehydrated to form a carotenoid containing liposomal formulation.

Meybeck et al. describe liposomal formulations comprising tretinoin or a retinoid, which is a structural analog of tretinoin. As appreciated by those versed in the art, retinoins, and specifically tretinoin, are **polar** substances, and as such are water-miscible. The present claims are directed to formulations containing **water-immiscible** carotenoids, which are quite different from the substances encapsulated by Meybeck et al.

In response to the Examiner's assertion that the same method steps are disclosed by Meybeck et al., it should be noted that Meybeck et al. disclose that the carotenoid and the lipid are mixed together: "2 g of soya lecithin and 0.1 g of tretinoin are dissolved in 30 ml dichloroethane in the presence of a lipophilic antioxidizing agent, *e.g.*, 0.0006 g alpha-tocopherol" (Example 1).

In contrast thereto, in the present invention it is **essential** that first the liposome-forming lipid be dissolved in the organic solvent in a level close to saturation, and only then is the carotenoid added. This order of mixing is required to obtain stable formulations. Meybeck et al. do not prescribe a specific order of adding the components to the

system, and thus cannot be regarded as novelty destroying with respect to the method claimed herein.

Claims 1-5, 7-19, 31-44, 46-47 and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by WO 94/13265, hereinafter Smith.

This rejection is respectfully traversed. Smith discloses on page 6, lines 17-39, that liposomes containing various combinations of the antioxidants can be prepared by methods known in the art …. For example, the liposomes can be made by dissolving a liposome forming compound or combination of such compounds in a suitable solvent. On page 7, lines 5-28, Smith discloses dissolving phospholipids or other liposome-forming compounds in a solvent and the solution placed into a suitable reaction vessel. The antioxidants are then dissolved in an aqueous solution, such as a buffered aqueous solution. However, the carotenoids used in the present invention are **water-immiscible**, and thus cannot possibly be dissolved in an aqueous solution.

Claims 1-19 are 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Stahl.

This rejection is respectfully traversed. Stahl et al. prepare liposomes by dissolving carotenoids, alpha-tocopherol or mixtures together with phosphatidylcholine in chloroform. The solvent was evaporated under a stream of

nitrogen. Multilammelar liposomes containing the respective compounds were prepared by adding 100 mM phosphate buffer to the lipid film, followed by sonication. This is not at all the same as the preparative method claimed herein. Stahl et al. describe formulations of multilamellar liposomes comprising carotenoids, alpha-tocopherol, or mixtures, dissolved with PC in chloroform. Stahl et al. dissolve the carotenoid **together with** the liposome-forming lipid (PC) followed by evaporation of the solvent.

The specification as filed demonstrates the superiority of liposomes prepared according to the method of the present invention. At page 25, lines 9-12, it is stated, "When lycopene encapsulated in MLV (ratio lipid/lycopene 10:1) was stored for three weeks at 4°C with soy oil covering them, the lycopene leaked from the liposome, and repartitioned in the oil phase, suggesting that lycopene can be transferred from the liposome to become bioavailable. However, the stability of lycopene in liposomes formulated according to formulations E1 to E8 of the present invention was determined, and the results shown in Table 5 on page 26 of the present invention. Tables 6A and 6B show the stability of different formulations of liposomes prepared according to the method of the present invention under different storage conditions.

Claims 20-26, 28-30 and 48-49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Meybeck et al.

This rejection is respectfully traversed. As noted above, Meybeck et al. dissolve loading of retinoids and specifically tretinoin on liposomes. These are **polar substances**, and thus one reading Meybeck et al. would not be able to encapsulate carotenoids in liposomes using this method, because the carotenoids would not dissolve in water. Table 6B of the instant application shows % degradation, which depends on the manner by which the components of the formulation are combined, *i.e.*, first dissolving the lipid in an organic solvent and only then adding a dry, water-immiscible carotenoid to the dissolved lipid. This order of addition of ingredients **is essential to obtain stable formulations.** The specification also provides comparative results showing that the formulations prepared by first dissolving the carotenoid and only then adding the dried phospholipids were less stable (as exemplified by formulations SO2 and SO4).

Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over Meybeck et al. in view of Mackaness. Mackaness teaches that organic solvents such as cyclohexane and chloroform could be used in dissolving the phospholipids.

This rejection is respectfully traversed. Even though Mackaness discloses that hexane can be used to dissolve phospholipids, combining Mackaness with Meybeck et al. would still not lead to the present invention. Meybeck et al. dissolve their retinoids in water to form liposomes. However, the present invention relates to carotenoids, which are not even miscible with water.

Claims 1-49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Meybeck et al. or Smith, further in view of Stahl et al.

This rejection is respectfully traversed. It is respectfully submitted that the cited publications in fact **teach away** from the method of the instant invention, as all of the cited publications, either alone or in combination, refer to preparative method steps in which the loading of the carotenoid into liposomes is either by dissolving both components together in a suitable solvent (as suggested by Meybeck et al. or Stahl et al.). or by dissolving the carotenoid in a suitable solvent and only then adding thereto the dried lipids, as suggested by Smith and Mackaness et al. The fact that none of the four publications even suggested the method steps of the present invention can only lead to the conclusion that it was not standard procedures, and could not have been obvious in light of these publications.

The preparative method claimed herein has been shown to produce liposomes which are more stable than liposomes prepared by the methods in the cited publications. All of the product claims now are product by process claims, since it has been demonstrated that the process of the present invention produces a superior product to those prepared by conventional methods.

All of the claims in the present application now relate either to a process for preparing the liposomes, or to liposomes and compositions containing these liposomes that are prepared by the herein claimed process. As demonstrated *supra*, the liposomes prepared by the method of the present invention are more stable than conventionally prepared liposomes, and therefore are not the same as the liposomes in the cited publications.

As noted above, none of the prior art preparative methods steps could have resulted in the loading onto liposomes of water-immiscible carotenoids. Meybeck et al. describe loading retinoids into liposomes, but these compounds are polar compounds. Smith described a method in which liposome forming compounds are dissolved in a solvent and then dried. An aqueous solution of the antioxidant is added to the dried material (see Example 7). As stated above, the carotenoids cannot be dissolved in an aqueous solution.

Additionally, there is a substantial advantage with respect to stability when preparing the formulations according to the present invention, as compared to the manner described by Smith.

Mackaness et al. Describe first hydrating the lipids in an organic solvent, drying the same, and then rehydrating the dry lipid film with a solution of the water soluble agent (column 3, lines 35-55). Thus, even had Mackaness succeeded in preparing liposomes loaded with water immiscible carotenoids, which is believed not to be the case, the formulations obtained are expected to be less stable than those obtained by the instant invention.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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